

## CASE REPORT

# Copper Poisoning in a Flock of Sheep. Copper Excretion Patterns after Treatment with Molybdenum and Sulfur or Penicillamine

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### SUMMARY

During an outbreak of chronic copper poisoning, fecal and urinary copper excretion were measured following treatment with molybdenum and sulfur supplementation of the feed (0.1 g ammonium molybdate plus 1 g sodium sulfate/sheep/day) or oral penicillamine (50 mg/kg bodyweight/day) using rams in metabolism cages. Serum glutamic-oxaloacetic transaminase activities and liver levels of molybdenum and copper in sheep that died were also monitored. Within four days of starting molybdenum and sulfur supplementation a highly significant increase in fecal copper excretion was evident and the increase persisted throughout the monitoring period (five weeks — general treatment of the flock continued for another three weeks). There was no effect of the molybdenum and sulfur supplementation on urinary excretion of copper. The molybdenum and sulfur supplementation was very effective, resulting in a rapid marked decrease in mortality. Oral penicillamine treatment induced cupruresis but did not affect fecal copper excretion. The results indicated that, while the cost of penicillamine may be a limiting factor for general treatment of a flock, it may be the drug of choice for the therapy of valuable breeding animals because cupruresis may be accurately and individually controlled. Serum glutamic-oxaloacetic transaminase activities were a valuable aid in diagnosing chronic copper toxicosis as well as for monitoring recovery. High initial liver copper levels were gradually reduced following molybdenum and sulfur treatment. However, at the end of the

study the liver copper levels of dead sheep varied within wide limits and there were still some sheep with high liver copper levels.

**Key Words:** Sheep, copper poisoning, copper excretion, serum transaminase activities.

### RÉSUMÉ

**Profil de l'excrétion du cuivre, après un traitement au molybdène et au soufre, ou à la pénicillamine, dans un troupeau de moutons aux prises avec un empoisonnement par cet élément**

Après avoir ajouté à la ration individuelle quotidienne des moutons d'un troupeau aux prises avec un empoisonnement chronique par le cuivre, 0,1 g de molybdate d'ammonium et 1 g de sulfate de sodium, ou après leur avoir administré une dose orale quotidienne de 50 mg de pénicillamine/kg, les auteurs placèrent six béliers dans autant de cages à métabolisme, afin de déterminer le profil de l'élimination fécale et urinaire du cuivre. Ils vérifièrent aussi l'activité de la glutamate-oxaloacétate-transaminase et la teneur du foie en molybdène et en cuivre, chez les moutons qui succombèrent à l'intoxication. Quatre jours après le début du traitement au molybdène et au soufre, on enregistra une très significative augmentation de l'excrétion fécale du cuivre; elle persista tout au long de la période de surveillance qui dura cinq semaines, alors que le traitement général du troupeau se continua pendant trois semaines additionnelles. Le traitement au molybdène et au soufre n'exerça aucune influence sur l'excrétion urinaire du cuivre, mais il se révéla

très efficace et provoqua une diminution rapide des mortalités. L'administration buccale de pénicillamine entraîna l'élimination du cuivre par l'urine, sans toutefois en affecter l'excrétion dans les fèces. Les résultats de cette étude révélèrent que, même si le coût élevé de la pénicillamine est susceptible d'en limiter l'utilisation pour traiter tout un troupeau, elle peut représenter la drogue par excellence pour le traitement de sujets reproducteurs de valeur, à cause de la possibilité d'un contrôle individuel et adéquat de l'élimination du cuivre par l'urine. L'activité de la glutamate-oxaloacétate-transaminase se révéla une aide précieuse pour le diagnostic de l'intoxication chronique par le cuivre, ainsi que pour la surveillance de l'évolution de la convalescence. La concentration hépatique initiale élevée de cuivre diminua graduellement, à la suite du traitement au molybdène et au soufre. À la fin de l'étude, on réalisa cependant que la concentration en cuivre du foie des moutons qui avaient succombé à l'intoxication variait beaucoup et que certains des survivants en affichaient encore une teneur élevée.

**Mots clés:** moutons, empoisonnement par le cuivre, excrétion du cuivre, activité de la glutamate-oxaloacétate-transaminase.

### INTRODUCTION

During the period from June 1981 to end of July 1981 chronic copper poisoning was diagnosed in the housed flock of sheep at the Animal Research Centre (ARC) in Ottawa. Levels of copper in liver up to 500 ppm, dry

matter basis (DM), are considered to be normal for sheep (1). If liver copper content exceeds 800 ppm there is a serious risk of hypercuprosis with the possibility of stress induced hemolytic crisis if steps are not taken to reduce the dietary levels of copper. Chronic copper poisoning in sheep occurs under conditions of moderate copper intake along with low dietary levels of molybdenum and sulfur (2,3,4). Hui-singh *et al* (5) reported that a copper-molybdenum (Cu-Mo) complex may be formed which either inhibits copper transport to liver cells or interacts through metabolic antagonism to prevent synthesis of ceruloplasmin and copper storage molecules. Molybdenum can impair copper utilization if the diet is relatively rich in sulfur (6). This interrelationship between copper, molybdenum and sulfur reduces the biological availability of copper and molybdenum in the rumen by forming copper-molybdenum complexes such as  $\text{Cu-MoO}_4$ . Sulfur and Mo are converted to thiomolybdate,  $\text{MoS}_4$ , which can readily be converted into insoluble Cu thiomolybdate,  $\text{CuMoS}_4$  (7,8). If molybdenum or sulfate are present in the diet in excess, copper absorption from the gastrointestinal tract drops sharply (9,10). This effect is probably due to the reduced absorption of copper and its increased excretion from the body. At high dietary Mo concentration, conversion of  $\text{MoO}_4$  to  $\text{MoS}_4$  in the rumen leads directly or indirectly to the formation of Cu-Mo complexes and reduction in copper absorption (8). Copper homeostasis is regulated primarily by its excretion rather than by its intestinal absorption.

Because it is well established that increased dietary Mo and sulfur reduce the copper concentration in sheep livers, resulting in rapid reduction in death losses due to chronic copper poisoning (4,11,12), the ARC flock was treated by supplementing the feed with 0.1 g of ammonium molybdate and 1 g sodium sulfate per sheep per day (Mo-S).

Penicillamine, which mobilizes and gradually reduces tissue copper, markedly increases urinary excretion of copper when given orally to sheep (13). Penicillamine is known to be distributed in the extracellular and at least in part of the intracellular space and to be retained in some regions for

an extended period (14). According to Smith *et al* (15) penicillamine may enter the body tissues and liberate protein-bound copper by a reduction effect. The relatively high cost of penicillamine may be a limiting factor for the general treatment of sheep. However, it may be a drug of choice in the therapy of valuable breeding animals because cupruresis may be accurately and individually controlled. Therefore, excretion patterns following penicillamine treatment were studied in addition to the flock treatment with Mo-S supplementation.

Very little information is available concerning the excretion patterns of copper by sheep that have accumulated high tissue levels of copper. Therefore, during the treatment for, and recovery from, the chronic copper poisoning fecal and urinary copper excretion were monitored. Serum glutamic-oxaloacetic transaminase (SGOT) activities and the levels of copper and molybdenum in livers of sheep that died from copper toxicity were also monitored.

The results of the monitoring studies are presented in order to provide information that may be of use in establishing a practical and effective method of eliminating excessive amounts of copper from tissues of sheep affected with chronic copper poisoning.

#### HISTORY AND CLINICAL SIGNS

The sheep were of mixed breeding, produced during the development of synthetic sire and dam strains in the ARC controlled environment research program (16). The flock had been re-established by hysterectomy in order to eradicate maedi-visna (17) and was ten to 12 months of age.

The sheep had been fed a complete mixed diet consisting of 20% corn silage, 30% alfalfa silage and 30% concentrate, fortified with vitamins and minerals. Feed copper levels in March and July (preceding and during the outbreak) ranged from 9.8 to 13.6 ppm (dry matter basis). The mineral supplement used in the diet was then changed to one which contained no copper. Subsequently, feed copper levels ranged from 5.2 to 8.3 ppm.

Molybdenum and sulfur analyses had not been performed before the

ARC outbreak. However, the levels were assumed to be low, i.e. 0.1 ppm to 0.3 ppm for Mo and less than 0.1% for total S (18), because low levels were observed in feed samples analyzed after the Mo-S treatment had been discontinued. Thus, it appears that dogmatic assertions regarding toxic or safe levels of copper for sheep, without considering dietary molybdenum and sulfur levels, are extremely hazardous.

The clinical signs and the histopathological changes observed during this copper poisoning were similar to those reported by Bundza *et al* (19). Typical postmortem observations are illustrated in Figure 1. There were varying degrees of jaundice, particularly noticeable in the conjunctiva and abdominal fat (Figure 1, Plate 1). Advanced postmortem decomposition of the liver and black kidneys in gas-filled renal capsules were common (Figure 1, Plates 1 and 2). Hemorrhage into the subcutaneous and interstitial tissue of the neck and chest region was found routinely as was occasional hemorrhage on the intestines and diaphragm (Figure 1, Plate 3).

#### MATERIALS AND METHODS

All sheep used for the monitoring studies were part of the main flock and remained in the same barns throughout the studies.

##### *Excretion Studies*

The excretion studies were carried out using rams which were placed in molded fiberglass metabolism cages (20). Each ram was also fitted with a harness and plastic feces bag to enable the separate collection of feces and urine. Representative samples for analysis were obtained by taking a 10% aliquot, after thorough mixing, from the daily collection of feces or urine for each ram.

Six rams were used to monitor excretion patterns resulting from the Mo-S treatment. For the first three days they received the same diet fed to the rest of the flock *without* the Mo-S supplement. From the fourth day on, a "medicated" premix was added to the feed, which provided approximately 0.1 g of ammonium molybdate and 1 g of sodium sulfate (increased to 2 g on the fifteenth day) per sheep per day. Samples of the daily feces and urine



FIGURE 1. Typical postmortem observations. Plate 1: Jaundice in abdominal fat. Plate 2: Swollen, black kidneys. Plate 3: Hemorrhages in intestines and diaphragm.

voided by each sheep from days 1 to 9 were analyzed for copper. Thereafter, the feces and urine collections from each sheep were composited, over either four or seven day periods, for analysis.

Six different rams were used to monitor excretion patterns resulting from special treatment with D-penicillamine (Cuprimine, capsules containing 250 mg, Merck Sharp and Dohme, Montreal). They received the same feed as the rest of the flock, but *without* the Mo-S supplement, throughout the trial. From day 4 to day 11 each sheep received a daily administration of 50 mg D-penicillamine/kg of body weight. The daily dosage was split between morning and afternoon. Samples of the daily feces and urine from each sheep were analyzed for copper. The trial lasted 16 days: three days pretreatment, eight days during treatment and five days posttreatment.

#### *Serum Glutamic-Oxaloacetic Transaminase Activity*

Fifty-five sheep were selected at random for the purpose of monitoring SGOT activity. The selected sheep remained with the main flock in their regular pens throughout the sampling period. The first blood samples were obtained from the jugular veins on the day preceding the addition of the "medicated" Mo-S supplement to the feed. During the following six weeks additional blood samples were obtained at approximately two week intervals from all survivors of the selected group.

#### *Liver Copper Levels*

All sheep that died were necropsied. During necropsy, liver samples were frozen for subsequent copper and molybdenum analyses.

#### *Analytical*

Copper was determined by atomic absorption spectrophotometry (Perkin-Elmer Model 460) following wet digestion (21). A method described by Khan *et al* (22) was used to determine molybdenum. Serum glutamic-oxaloacetic transaminase activities were measured by the method of Reitman and Frankel (23).

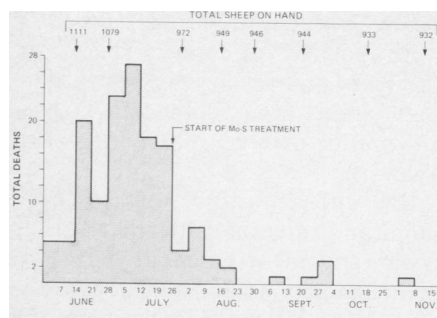


FIGURE 2. Weekly sheep mortality — copper related deaths.

### Statistical Analyses

The data for urinary and fecal excretion over time were examined by analyses of variance (24). Because of the relationship between means and variances, a logarithmic transformation of the data was used.

### RESULTS AND DISCUSSION

The size of the flock and the weekly mortality due to chronic copper poisoning before and after Mo-S treatment is shown in Figure 2. While the losses were high during the outbreak, Mo-S supplementation of the feed was very effective, resulting in an immediate and marked drop in losses.

#### Copper Excretion Following Dietary Supplementation with Molybdenum and Sulfate

An increase in fecal copper excretion became evident approximately four days after initiation of the Mo-S treatment (Figure 3) and persisted throughout the remainder of the monitoring period (approximately five weeks, general treatment of the flock continued for another three weeks). The analysis of variance (ANOVA) for the overall fecal copper excretion pattern showed highly significant ( $P < 0.01$ ) differences associated with time periods and for the comparison of before treatment with after treatment (Table I). It was noticed, however, that three of the six sheep had low, and the other three high, fecal copper excretions before treatment. An ANOVA including these two subgroups in the model indicated highly significant ( $P < 0.01$ ) differences for times and for the comparison of before and after Mo-S treatment (Table II). Interac-

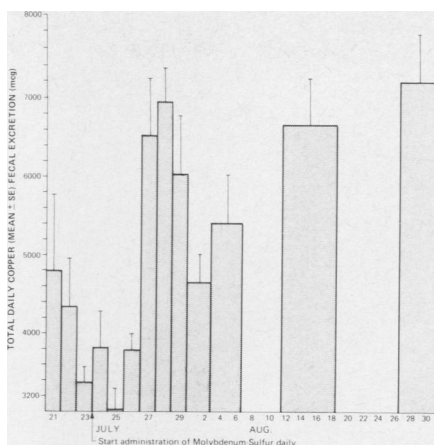


FIGURE 3. Fecal copper excretion before and after Mo-S treatment.

tions between group and time as well as between group and comparison were also highly significant ( $P < 0.01$ ). Separate ANOVA for each subgroup (Table III) showed that for both groups the differences due to time as well as the comparison before and after treatment were highly significant ( $P < 0.01$ ). Before Mo-S supplementation the mean daily copper excre-

TABLE III  
ANALYSES OF VARIANCE FOR TWO SUB-GROUPS HAVING HIGH OR LOW FECAL COPPER EXCRETION BEFORE Mo-S TREATMENT (DATA IN TRANSFORMED)

Source	df	MS	F
<b>Low Group</b>			
Sheep	2	0.0021	
Time	12	0.3587	10.34**
C <sup>a</sup>	1	2.0897	60.32**
Error	24	0.0347	
<b>High Group</b>			
Sheep	2	0.0033	
Time	12	0.2453	11.74**
C <sup>a</sup>	1	0.2217	10.61**
Error	24	0.0209	

<sup>a</sup>Comparison of before treatment with after treatment.

tions were 2928 and 5070  $\mu\text{g}$  for the low and high groups, respectively. After Mo-S supplementation mean daily copper excretions were 4832 (65% increase) and 5991  $\mu\text{g}$  (18% increase) for the low and high groups, respectively. These results agree with a previous report (25) that dietary Mo-S treatment to sheep increased the endogenous fecal excretion of stable copper. It has also been reported (26) that high dietary molybdenum for sheep promoted copper excretion in the feces by influencing the copper metabolism of rumen microorganisms resulting in a reduction in the proportion of soluble copper reaching the duodenum.

There was no effect of the Mo-S supplementation on urinary excretion of copper in this study. The findings for urinary copper excretion did not agree with previous reports. Administration of molybdenum to sheep has been reported to increase the urinary excretion of copper, but the amount of copper excreted in the urine was not related to the dose rate of molybdenum (27). It has also been observed (11,28) that in sheep supplemented with molybdenum-sulfate there was an increase in the volume of urine excreted, which could have been an important factor in the increase of total copper eliminated through the urinary tract. Smith *et al* (15), on the other hand, found that an increase in the dietary intake of molybdenum and sulfate resulted in an increase in the loss of endogenous copper from the body in both urine and feces.

TABLE I  
ANALYSIS OF VARIANCE FOR FECAL COPPER EXCRETION PATTERNS OF SIX SHEEP TREATED WITH Mo-S (DATA IN TRANSFORMED)

Source	df	MS	F
Sheep	5	0.3991	
Time	12	0.5276	14.07**
C <sup>a</sup>	1	1.8364	48.97**
Error	60	0.0375	

<sup>a</sup>Comparison of before treatment with after treatment.

TABLE II  
ANALYSIS OF VARIANCE FOR FECAL COPPER EXCRETION PATTERNS OF SHEEP HAVING HIGH OR LOW EXCRETION BEFORE Mo-S TREATMENT (3 SHEEP IN EACH GROUP, DATA IN TRANSFORMED)

Source	df	MS	F
Groups (G)	1	1.9849	
Sheep/G	4	0.0027	
Time (T)	12	0.5276	18.98**
C <sup>a</sup>	1	1.8364	66.06**
G x T	12	0.0764	2.75**
G x C	1	0.4750	17.09**
Error	48	0.0278	

<sup>a</sup>Comparison of before treatment with after treatment.

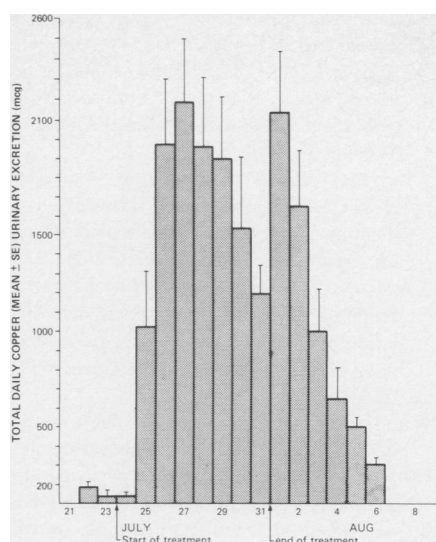


FIGURE 4. Urinary copper excretion before, during and after penicillamine treatment (mean  $\pm$  SE).

TABLE IV  
ANALYSIS OF VARIANCE FOR THE URINARY COPPER EXCRETION PATTERN RESULTING FROM PENICILLAMINE TREATMENT (DATA IN TRANSFORMED)

Source	df	MS	F
Sheep	5	2.1294	
Time	15	6.2494	28.41**
C1 <sup>a</sup>	1	64.4226	288.28**
C2 <sup>b</sup>	1	7.7076	32.76**
Error	70	0.2200	

<sup>a</sup>Comparison of period 1 (before treatment) with periods 2 (during) and 3 (after treatment).

<sup>b</sup>Comparison between periods 2 and 3.

#### Copper Excretion Following Penicillamine Treatment

Copper excretion following oral administration of penicillamine showed that this chelating agent induced cupruresis (Figure 4). When the treatment was discontinued, the cupruresis reduced gradually over the final six days of the study. Statistical analysis of the data (Table IV) showed highly significant ( $P < 0.01$ ) differences in urinary copper excretion due to time, and in excretion during (period 2) and after (period 3) treatment compared with before (period 1) treatment. The mean decrease in urinary copper for period 3 compared with period 2 was also highly significant ( $P < 0.01$ ). On the other hand, as was expected, penicillamine treatment of the copper-loaded sheep did not affect

TABLE V  
COPPER  $\mu\text{g/g}$  DRY MATTER AND MOLYBDENUM  $\mu\text{g/g}$  DRY MATTER IN THE LIVERS OF SHEEP THAT DIED FROM COPPER TOXICITY (MEANS  $\pm$  SE)

	Mid July	August	September	October
Copper	1251 $\pm$ 296 (10) <sup>a</sup>	1159 $\pm$ 348 (15)	872 $\pm$ 413 (8)	745 $\pm$ 334 (8)
Molybdenum	2.23 $\pm$ 0.88 (6)	2.33 $\pm$ 0.97 (8)	3.94 $\pm$ 23.13 (5)	4.70 $\pm$ 1.13 (8)

<sup>a</sup>Figures in parenthesis indicate number of samples.

TABLE VI  
NUMBERS OF SHEEP HAVING SERUM GLUTAMIC-OXALOACETIC TRANSAMINASE ACTIVITIES LESS THAN OR MORE THAN 200 FRANKEL UNITS (FU)<sup>a</sup>

FU	July 20	Aug. 5	Aug. 20 <sup>b</sup>	Sept. 3
< 200	21	39	43	49
> 200	34	14	7	2

$\chi^2 = 51.05$ , 3df,  $P < 0.0001$ .

<sup>a</sup>Less than 200 FU are considered as normal whereas values over 200 FU are considered as definitely abnormal (30).

<sup>b</sup>No sample from one animal.

TABLE VII  
FREQUENCY DISTRIBUTION OF OBSERVED SERUM GLUTAMIC OXALOACETIC TRANSAMINASE ACTIVITIES

Range of SGOT values	July 20	Aug. 5	Aug. 20 <sup>a</sup>	Sept. 3
0 - 200	21	39	43	49
201 - 499	23	9	7	2
501 - 700	6	2		
701 - 1000	3			
> 1000	2	3		
Totals	55	53	50	51

<sup>a</sup>No sample from one animal.

fecal copper excretion. These results from the present study are in agreement with the previous report (13) that penicillamine given orally to sheep rapidly enhanced the urinary excretion of copper (within 24 h after dosing and by factor of 10).

#### Copper and Molybdenum Levels in Liver

Hepatic concentrations of copper decreased throughout the period of the study, whereas hepatic molybdenum concentrations increased (Table V). The analysis of variance of the data showed both trends to be highly significant ( $P < 0.01$ ). The results of this study were in agreement with those reported by previous workers (28) who also showed a gradual reduction in high liver copper coinciding with a marked decrease in sheep mortality due to cuprotoxicity following Mo-S treatment. However, at the end of the

ARC copper poisoning outbreak the copper concentrations in the livers of dead sheep varied within wide limits and there were still some sheep with high hepatic copper levels despite the Mo-S treatment. However, the Mo-S treatment was considered highly effective because of the dramatic reduction in death losses due to chronic copper poisoning.

#### Serum Glutamic-Oxaloacetic Transaminase Activity

Of the original 55 selected sheep, two died during the first two weeks and two during the second two weeks. However, there did not appear to be a relationship between the four deaths and previous SGOT levels (200, 2300, 270 and 1500, respectively). The remaining results (Tables VI and VII) indicated that the measurement of SGOT activity was a valuable aid in diagnosing this chronic copper poison-

ing. The decreases ( $P < 0.0001$ ) in SGOT, which occurred in this study following the Mo-S treatment, also attest to the importance of the use of this simple serum enzyme assay for monitoring the recovery following removal of the primary factor which affects liver function. It is proposed that the decreased activity of SGOT following treatment may reflect changes in liver metabolism associated with a decrease of the excessive amounts of copper stored in the liver. From the results presented here, as well as previous reports (12,29), it is clear that the serial estimation of this enzyme could be a useful diagnostic tool as well as an indicator of the effectiveness of treatment against chronic copper poisoning in sheep.

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